SOURCES OF ENTEROTOXIGENIC STAPHYLOCOCCI

BY

ESTHER MEYER

B.S., University of Michigan, 1930
M.S., University of Illinois, 1933

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1944

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As early as 1894 an outbreak of food poisoning, probably caused by staphylococci, was reported. Following this, scattered reports appeared in the literature, but not until 1930 were staphylococci recognized as an important cause of food poisoning.

The clinical symptoms of staphylococcal food poisoning include a short incubation period (usually one to four hours after consumption of the contaminated food), pain in the abdomen, dizziness, prostration, pallor, and cold sweats. Repeated vomiting attacks generally occur for three to five hours or longer. Following this, recovery is usually rapid, the patients feeling quite normal in twenty-four to forty-eight hours. However, some may experience anorexia and general weakness for several days. A few fatalities have been reported.

The majority of cases result from the ingestion of custard bakery goods; however, milk and milk products, various kinds of meats, including the pickled and smoked types, such as tongue and ham, chicken, fish, and gravies have all been incriminated.

It was shown in 1930 that in foodstuffs the presence of a preformed toxic metabolite of staphylococci suitably called "enterotoxin" was responsible for the gastrointestinal symptoms. At first human volunteers and later monkeys were used to test the potency of culture filtrates by oral administration. In both, considerable variation in susceptibility was encountered. Because of this, intra-abdominal or intravenous injection of kittens and cats was suggested as the possible solution to the problem of finding a suitable experimental animal. In this work kittens and *Daphnia magna,* a minute crustaceous animal were used to test the potency of culture filtrates.

A controversy regarding the specificity of the kitten test has recently appeared in the literature. Most investigators believe enterotoxin to be a separate toxic entity while a few others believe alpha and beta hemolysins may both cause gastrointestinal symptoms in these animals. Beta hemolysin, the more thermostable of the two, is considered to be identical with enterotoxin.

While a number of outbreaks have been reported, the actual incidence is unknown, because only when large groups of people are involved is the matter brought to the attention of public health authorities. The geographical distribution of these outbreaks is of interest in that practically all have been in the United States and Canada, where it is now regarded as the commonest form of food poisoning. Recently a few outbreaks occurred in the British Isles.

The sources of staphylococci responsible for food poisoning are of con-

siderable epidemiologic interest. That there is need for new surveys of the incidence of these strains, which may possibly reveal some degree of selective distribution, has frequently been expressed.

In this investigation a survey was conducted in the hope that the information obtained would be of value in the control of this type of food poisoning.

The results are tabulated below:

Sources of Strains	*No. Tested*	*No. of Kitten-Positive Strains*	*Sources of Strains*	*No. Tested*	*No. of Kitten-Positive Strains*
Normal mouth	9	4	Infected wounds	4	1
Mastitis in cattle	8	4	Chicken-joint lesions, skin lesions, and staphylococcosis	8	3
Normal nose	13	2	Boils and abscesses	9	2
Air	9	4	Septicemia	1	0
Impetigo	11	8	*Totals*	93	32
Sore throats	10	3			
Normal skin	11	1			

As the table shows, 34.4% were positive.

Hemolysin Studies

Filtrates prepared from forty-seven of the ninety-three cultures of staphylococci were assayed for alpha and beta hemolysins. Eighty-two per cent of the kitten-positive cultures produced alpha hemolysin only. Two oral strains produced alpha and beta, one isolated from a chicken suffering from staphylococcosis produced beta predominantly, and one failed to elaborate soluble hemolysins.

Eighty-three per cent of the kitten-negative cultures produced alpha hemolysin only. One strain produced alpha and beta and three strains failed to elaborate soluble hemolysins.

Relationship Between Enterotoxin and Hemolysins

It was found that beta hemolysin is capable of evoking a gastrointestinal syndrome in kittens, but it differs from that produced by enterotoxin and is readily distinguishable.

Whenever filtrates containing alpha hemolysin produced gastrointestinal symptoms, enterotoxin was found to be associated with it; alpha hemolysin alone did not produce symptoms.

From the results obtained in this work, the kitten is a satisfactory laboratory animal for demonstrating the presence of enterotoxin regardless of whether the intraperitoneal or intravenous route is used. The latter is the less convenient of the two methods, and it does not seem to offer sufficient advantages to recommend its use routinely. However, it is well to determine hemolysins present in the filtrates before subjecting them to this biological test.

Complete evacuation of the intestinal tract in control Daphnia required an average of one hour and fifteen minutes. Enterotoxic filtrates by increasing peristalsis and the magnitude of the peristaltic waves brought about complete evacuation in fifteen to twenty minutes.

Beta hemolysin stimulated the most active peristalsis, evacuation occurring in five to ten minutes. Even when the same filtrates were heated until no beta hemolysin was detectable, evacuation occurred in fifteen minutes. The explanation of this is not clear, but it is possible that the hemolytic properties of beta hemolysin are destroyed more rapidly than its capacity to produce reactions in animals.

SUMMARY

The results of this work indicate that staphylococci on the skin and in the normal nose do not constitute as great a hazard as those found in the normal oral cavity and air. Those found in the air most likely have their origin in the mouth.

Certain pathological conditions, such as impetigo, mastitis in cattle, sore throats, infected wounds, boils, and abscesses are sources of these strains. It is admittedly beyond one's power to control natural existing conditions, but it is obvious that persons suffering from staphylococcic infections should not be permitted to handle perishable foodstuffs.

Staphylococcic infections in chickens are possibly another common source of these organisms, but it would be difficult to suggest a control method, except to recommend thorough cooking and subsequent refrigeration.

This investigation would indicate a wide distribution of enterotoxigenic staphylococci and a fairly high incidence, thirty-two out of ninety-three strains, or 34.4 per cent.

The kitten test was found to be a satisfactory method for the identification of enterotoxin. Beta hemolysin will elicit a similar reaction in this animal.

Daphnia magna may be used as the test animal, but is limited to those filtrates containing either alpha hemolysin only or alpha hemolysin plus enterotoxin. Because of this and other factors, such as the culturing of Daphnia, they are perhaps not as useful as the kitten.

No completely satisfactory *in vitro* tests are available at the present time to differentiate food poisoning strains from others. Stone's medium was not sufficiently specific to be useful. Chapman's tests, while not specific, are helpful in selecting strains which are likely to produce enterotoxin.

VITA

The writer was born in Chicago, Illinois, October 1, 1902. She received her primary, secondary, and junior college education in the public schools of Chicago, Illinois.

In September, 1922, she entered the University of Illinois College of Pharmacy and was graduated in June, 1924, with the degree of Ph.G. In September, 1924, she joined the faculty of the same college as Assistant in Bacteriology.

The writer was enrolled in the University of Michigan, College of Pharmacy, for summer sessions from 1926 to 1929 inclusive and one residential year (1929-1930). In June, 1930, she received the degree of Bachelor of Science in Pharmacy. Later, in June, 1930, she entered the Graduate School enrolling in advanced courses in bacteriology. At the close of the summer session, she returned to the University of Illinois College of Pharmacy, as Instructor in Bacteriology.

In September, 1930, she entered the University of Illinois Graduate School, Chicago campus, with a major in bacteriology and received the degree of Master of Science in October, 1933.

In September, 1937, the writer was given an increase in rank to Associate in Bacteriology in the University of Illinois College of Pharmacy. This department became part of the Department of Bacteriology and Public Health in the College of Medicine in 1941, and at this writing she holds the same rank in the latter college.

She is a member of the following societies: Rho Chi, Sigma Xi, The Society of Illinois Bacteriologists, and the American Pharmaceutical Association.

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